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## 1941

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PROCEEDINGS

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# A NOTE ON CURVES CONGRUENT TO THEIR EVOLUTES

By S. M. Kerawala

Department of Mathematics, Muslim University, Aligarh

Communicated by Sir Shah Sulaiman

(Received on November 13, 1940)

1. If $c_1$ is the evolute of a plane curve $c$, $c_2$ that of $c_1$, $c_3$ that of $c_2$ and so on, assuming the curve $c$ to be given in its intrinsic form $\rho=\phi(s)$, Pirondini[1] has found the necessary and sufficient conditions which $\phi(s)$ must satisfy in order that $c$ be congruent to $c_r$. In this note, I obtain equivalent conditions, but in a more compact form.

2. $\rho, \rho_1, \rho_2, \ldots\ldots$ will denote the radii of curvature, $s, s_1, s_2, \ldots\ldots$ the arc-lengths of $c, c_1, c_2, \ldots\ldots$ and $\psi, \psi_1, \psi_2, \ldots\ldots$ the angles which the tangents at corresponding points of $c, c_1, c_2 \ldots$ make with a fixed line in the plane. If $c$ has the form

$$s=f(\psi),\ \rho=f'(\psi),$$

where the prime signifies differentiation with respect to $\psi$, then $c_r$ has the form

$$s_r=f^{(r)}(\psi),\ \rho_r=f^{(r+1)}(\psi).$$

If $c_r$ is congruent to $c$, it must be of the form

$$s_r=f(\psi_r),\ \rho_r=\frac{d}{d\psi_r}f(\psi_r)$$

But $d\psi_r=d\psi$, hence $\psi_r=\psi+\alpha_r$ where $\alpha_r$ is a constant. Comparing the two forms of $c_r$, we have at once the theorem :

A necessary and sufficient condition that $c_r$ be congruent to $c$ is that a constant $\alpha_r$ should exist such that

$$f^{(r)}(\psi)\equiv f(\psi+\alpha_r) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

3. (1) is a particular form of a general difference—differential equation considered by Schmidt[2] and Titchmarsh.[3] For the purpose of this problem, Schmidt's method is the more suitable for application. Using this method, I obtain simple solutions of the problem for $r=1$.

If $r=1$, (2) assumes the form

$$f'(\psi)=f(\psi+\alpha_1) \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

If $\alpha$ be written for $\alpha_1$ and $p+iq=m$, where $\alpha$, $p$, $q$ are real, $e^{m\psi}$ is a solution of (2) provided

$$\left.\begin{aligned} p&=e^{\alpha p}\cos \alpha q,\\ q&=e^{\alpha p}\sin \alpha q \end{aligned}\right\} \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

If any arbitrary value be assigned to $\alpha$, there will be an infinity of values of $p$ and $q$ which will satisfy (3). Calling these solutions $p_i$, $q_i$ $(i=1, 2, \ldots)$, the general solution of (2) associated with the particular value chosen for $\alpha$ will be

$$f(\psi)=\sum_i c_i\ e^{p_i\psi}\cos(q_i\psi+\varepsilon_i) \quad . \quad . \quad . \quad . \quad (4)$$

where $c_i$, $\varepsilon_i$ are arbitrarily chosen real constants.

I consider now particular solutions of (3) by assigning simple values to $\alpha$ and restricting the freedom of $p$ and $q$ by one condition.

(*i*) $q=0$, $0<\alpha<\frac{1}{e}$. The solution can be shown to be

$$f(\psi)=C_1\ e^{p_1\psi}+C_2\ e^{p_2\psi},$$

where $p_1>e$, $1<p_2<e$, $\frac{\log p_1}{p_1}=\frac{\log p_2}{p_2}=\alpha$, and $C_1$, $C_2$ are arbitrary constants. The $s-\rho$ equation of this curve is

$$B_1\ (sp_1-\rho)^{p_1}=B_2\ (\rho-sp_2)^{p_2},$$

where $B_1$, $B_2$ are new arbitrary constants. If either $B_1$ or $B_2$ vanishes, we have at once the logarithmic spiral

$$\rho=ps \qquad (1<p)$$

(*ii*) $\alpha=0$. There is only one solution, *viz.*

$$f(\psi)=C\ e^{\psi},$$

yielding the logarithmic spiral $\rho=s$.

(*iii*) $q=0$, $\alpha<0$. Again, there is only one solution, namely,

$$f(\psi)=C\ e^{p\psi} \qquad (p<1)$$

and again the $s-\rho$ equation is

$$\rho=ps \qquad (p<1)$$

From (*i*), (*ii*) and (*iii*) it follows that any logarithmic spiral is congruent to its evolute, a theorem first proved by Johann Bernoulli.[4]

(*iv*) $q=0,\ \alpha=\frac{1}{e}$.

The solution runs

$$f(\psi)=(C_1\psi+C_2)\,e^{e\psi},$$

a curve of which the $s-\rho$ equation is

$$(\rho-es)=C\,e^{\frac{\rho}{\rho-es}}$$

(*v*) $p=0,\quad \alpha=\frac{\pi}{2}$. In this case the solution is

$$f(\psi)=C_1\cos\psi+C_2\sin\psi,$$

a curve having for its $s-\rho$ equation

$$\rho^2+s^2=a^2$$

This is the cycloid, first found to be congruent to its evolute by Huygens.[4]

(*vi*) $p=q$. The solution is

$$f(\psi)=C\,e^{p\psi}\cos p\psi, \qquad (\ p\sqrt{2}=e^{\pi/4}\ )$$

yielding as the $s-\rho$ equation the result

$$\log[\,p^2s^2+(ps-\rho)^2\,]-2\tan^{-1}\frac{ps-\rho}{ps}=A \quad \text{where} \quad p=\frac{1}{\sqrt{2}}\,e^{\pi/4}.$$

The six cases considered above illustrate the main types of the simplest possible solutions. The construction of more complicated solutions presents no difficulty.

4. In a like manner, the curves which are congruent to their higher evolutes can be discussed.

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# DIRECTIONAL ASYMMETRIES IN COSMIC RADIATION AT LAHORE

By Piara Singh Gill

Forman Christian College, Lahore

(Received on December 18, 1940)

SUMMARY

Four triple-coincidence cosmic ray telescopes, directed at a common zenith angle of 60°, were mounted on a turn-table. Each telescope represented a vertical plane 90° from that of its neighbour. In each was inserted 10·2 cms. of lead. By automatic 180° reversals every 35 minutes, each telescope interchanged its position with the one directly opposite. Countings were made at settings of the table for every ten degrees of azimuth angle. N-S and E-W differences have been found and they agree fairly well with the findings of previous investigators and with the Lemaitre-Vallarta theory for these differences. The azimuthal variations in N-W quadrant is about 6%, the intensity being greater as one goes from north to west.

The theory of the effect of the earth's magnetic field on cosmic rays as worked out by Lemaitre and Vallarta and their collaborators predicts a variation of cosmic ray intensity at a given point on the earth's surface and fixed zenith angle, but for different azimuths (azimuthal effect) special cases of this are the north-south and eastwest asymmetries. Rossi[1] was the first to determine the east-west asymmetry both experimentally and theoretically.

The experiments performed by various investigators at different latitudes and altitudes give greater intensity from the west which proves that positive primaries contribute more to the intensity than do primary negatives. The east-west effect depends on the difference between positives and negatives. North-south effect was established by the experiments, one by Johnson[2] in Mexico (geomagnetic latitude 29°N) and the other by Clay[3] in Java (geomagnetic latitude 18°S). These experiments show that in the northern geomagnetic hemisphere the intensity of cosmic radiation in the geomagnetic meridian is, for equal zenith angles, greater from the south than from the north; conversely in the southern hemisphere it is greater from the north than from the south. The north-south effect does not depend on the sign of the primary particles.

Since the north-south asymmetry depends upon the sum of positives and negatives, whereas the east-west asymmetry depends on their difference, a comparison between them gives valuable information as to the ratio of positives and negatives in a given energy range. The present experiment at Lahore (geomagnetic latitude 22°N) though primarily carried out to find the azimuthal

variation of cosmic radiation at a fixed angle of 60°, gives the north-south and east-west asymmetries as special cases. Because of the large variation of the intensity with zenith angle brought about by atmospheric absorption, it is important in measuring the geomagnetic directional effects to compare intensities only in directions for which the zenith angle is constant.

*Experimental arrangement.*—Four cosmic-ray telescopes, each consisting of three triple coincidence G-M counter tubes with lead of 10·2 cm. to filter out soft component of cosmic radiation. Distance between the centres of the outer tubes is 20 cm. in each case. The solid angle range covered by each telescope is 14·5° in the verticle and 65° in the lateral planes. The telescopes are mounted on four edges of square table which is rotated automatically back and forth through an angle of 180° so that opposite telescopes interchange positions at regular intervals. One telescope stays in one position for 35 minutes. The connections to the recorders are automatically changed, so that each counts rays from one direction only. The interchanging of opposite telescopes at regular intervals automatically corrects for the inevitable differences in counting rates of these two sets. Cosmic-ray intensities from two directions can be compared with an accuracy depending only upon the statistical fluctuations of the total number of rays counted, provided the apparatus is shifted back and forth between the two opposite directions at frequent intervals.

The apparatus is installed in a special observatory constructed for the purpose on top of the new physics laboratory of Forman Christian College. The roof of the observatory is made of a single sheet of galvanized iron and is thus vertically open to the sky.

Results.

| Directions | N | S | E | W |
|---|---|---|---|---|
| Total Nos. of counts | 1747 | 1960 | 2303 | 2446 |
| Counts per minute | 0·415 ± ·007 | 0·466 ± ·007 | 0·412 ± 066 | 0·438 ± ·006 |

In the table are given the total number of counts from each of the four directions, north, south, east and west at a fixed zenith angle of 60°. The total time of counting from north and south was 4200 minutes in each case, while from east and west the total time was 5600 minutes. Numbers of counts per minute are also given with their probable errors.

The east-west and north-south differences in the count rate in per cent are 6·03 and 11·50 respectively. The north-south asymmetry for various latitudes and for different zenith angles was theoretically studied by Lemaitre and Vallarta.[4] Their value for 60° zenith angle at 20° magnetic latitude, calculated from their curves, is found to be 12·7 per cent. The difference in the theoretical and experimental value is partly due to the difference in the latitude of Lahore (22° magnetic) and the latitude for which the calculations were made. The east-west difference of

6·03 per cent at Lahore can only be compared with the experimental value (3·5) per cent. of Johnson[5] at 20° magnetic latitude at Panama. Johnson did not use any lead while in the present experiment 10·2 cms. of lead is inserted in the cosmic-ray telescope This result indicates that the asymmetry in the east-west directions at 60° zenith angle is greater for hard component of cosmic rays.

The author wishes to express his gratitude to the University of Chicago for granting him a research fellowship and supplying the apparatus. The author also takes pleasure in acknowledging a grant by Sir Dorabji Tata Trust, which has made possible the carrying out of this research. Forman Christian College has offered the facilities of the Physics Department where the work is being carried on.

*References*

1. B. Rossi, *loc. cit.*, ref. 7.
2. T. H. Johnson, Phys. Rev., **47**, 91 (1935).
3. J. Clay, Physica, **8**, 867 (1935).
4. G Lemaitre and M. S. Vallarta, Phys. Rev., **49**, 719 (1936).
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# COMPOSITION OF PATENT STILL MOLASSES FUSEL OIL OF INDIAN ORIGIN, PART IV

By Sikhibhushan Dutt

Chemistry Department, University of Allahabad

(Received on November 26, 1940)

SUMMARY

Indian Patent Still molasses fusel oil obtained from the Mandya Distillery, Mysore, through the courtesy of the Government of Mysore, was submitted to exhaustive fractional distillation, whereby it was resolved into the following constituents:

| | |
|---|---|
| Ethyl alcohol | traces |
| Isopropyl alcohol | 18·4 per cent |
| n-Propyl alcohol | 0·49 „ „ |
| Water | 5·1 „ „ |
| Acetal | 0·16 „ „ |
| Isobutyl alcohol | 0·16 „ „ |
| Ethyl isobutyrate | 0·18 „ „ |
| n-Butyl alcohol | 0·34 „ „ |
| Isoamyl alcohol | 66·2 „ „ |
| n-Amyl alcohol | 0·71 „ „ |
| Ethyl valerianate | 0·02 „ „ |
| n-Hexyl alcohol | 0·07 „ „ |
| n-Heptyl alcohol | 0·32 „ „ |
| n-Octyl alcohol | 0·51 „ „ |
| n-Nonyl alcohol | 0·06 „ „ |
| Higher esters | 2·30 „ „ |

In three previous communications by the present author,[1,2,3] molasses fusel oils from the Patent Still Distilleries of Messrs Carew & Co. Ltd. of Shahjehanpur, Messrs Begg Sutherland & Co. Ltd. of Cawnpore and of Messrs Muree Brewery Co. Ltd. of Rawalpindi were submitted to intensive fractional distillations with the help of special fractionating apparatus and ultimately separated into a large number of constituents consisting mainly of alcohols and esters. In the present investigation, molasses fusel oil obtained from an entirely differnt source, and one situated at the southern end of India, namely, the Mandya Distillery, Mysore, was taken for examination. In this Patent Still Distillery which is adapted for the continuous

production of Power Alcohol by the azeotropic process, fusel oil also is produced continuously although in small quantities, but this is not recovered for want of methods for its proper utilisation. This fusel oil on examination was found to be very similar to the Rosa fusel oil in the fact that it contained a large proportion of isopropyl alcohol. It was also similar to the Cawnpore fusel oil inasmuch as it contained a comparatively large proportion of high-boiling esters. The waxy matter that separated from the fusel oil on standing and also during the progress of the distillation, was found to have a composition identical with that of Rawalpindi fusel oil. It melted at 125-126°C and gave lauric acid on hydrolysis.

## Experimental

Mandya fusel oil is a bright yellow mobile liquid with a specific gravity of 0·839 at 37°C. It has a characteristic empyrheumatic smell reminding of old rum. The oil on allowing to stand for some time at the ordinary temperature (35°C) deposited a small quantity of dirty white crystalline wax. Further quantities of the same material were obtained during the progress of the distillations. The total quantity of the crude stuff available for examination was however less than one gram (0·72g.). The total quantity of fusel oil taken for distillation was about four gallons (17720 c.c.).

The fractional distillation of the fusel oil was done in the same manner as in the cases of the three previous fusel oils already described in published papers. The final results have been given in the summary. The high-boiling esters obtained as tail fraction of the distillations to the extent of 2·3 per cent of the entire fusel oil, were further examined and resolved into the following constituents:

| | |
|---|---|
| Ethyl caprate | 0·23 per cent |
| Isoamyl-n-octylate | 0·72 „ „ |
| n-Amyl pelargonate | 0·32 „ „ |
| n-Amyl caprate | 0·43 „ „ |
| n-Octyl caprate | 0·23 „ „ |
| n-Amyl laurate | 0·04 „ „ |
| n-Amyl myristate | 0·02 „ „ |
| Unidentified | 0·31 „ „ |
| | Total = 2·30 „ „ |

The dirty white waxy matter that had separated out from the fusel oil was repeatedly crystallised from absolute alcohol, when it was obtained in the form of large rectangular plates with fatty lustre, melting at 125-126°C. The melting point was undepressed on mixing with the wax obtained from Rawalpindi fusel oil melting

at the same temperature. It is therefore identical in composition with the latter substance.

I take this opportunity in expressing my best thanks to the Mandya Distillery Co., Ltd., Mysore, and also to the Director, Industrial Research Bureau, Calcutta, for kindly arranging to send me 4 gallons of decanted fusel oil.

*References*

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2. Dutt, S. (1939), Proc. Nat. Acad. Sci., 9, 69.
3. Dutt, S. (1940), Proc. Nat. Acad. Sci., 10, 113.

# A THEORY OF COLOUR ON THE BASIS OF MOLECULAR STRAIN PART VIII* COLOUR IN RELATION TO CHEMICAL CONSTITUTION OF ORGANIC NITROSO AND ISONITROSO COMPOUNDS

By Sikhibhushan Dutt

Chemistry Department, Allahabad University

(Received on November 26, 1940)

SUMMARY

1. In accordance with a Theory of colour on the basis of molecular strain advanced by the present author, the true nitroso (N = O) group is the most fruitful source of intense colour amongst organic compounds due to the high degree of molecular strain contained therein.

2. Due to the above reason, the nitroso group has always a tendency to lose the internal strain by oxidation, reduction or by the formation of an isonitroso compound by migration of a labile hydrogen atom in vicinity.

3. But under certain circumstances, a weakly strained isonitroso compound may pass into a highly strained nitroso compound with consequent development of intense colour, by the same process of migration of a hydrogen atom, whereby an oximino-ketonic structure gets converted into a nitroso-enolic structure.

4. The above structural change takes place by the action ofalkalis and organic bases, whereby the weakly acidic oximino-ketonic form becomes converted into a strongly acidic nitroso-enolic form, which later becomes stabilised by salt formation.

5. From a study of a large number of isonitroso compounds, the present author has come to the conclusion that wherever there are a number of possibilities of tautomerism of the oximino-ketonic form into the nitroso-enolic form, the greatest development of colour is produced.

In accordance with a theory of colour on the basis of molecular strain advanced by Dutt,[1] the visible colour of substances is due to selective absorption of certain electromagnetic vibrations causing the sensation of white light and the transmission of the remainder. The cause of selective absorption of any organic compound lies in its molecular strain, such strain being imparted to the molecule by the distortion of the normal valency directions of atoms produced by any of the following causes :—

(1) Formation of a double or triple bond.

(2) Formation of a cyclic from an open-chain compound.

(3) Unequal distribution of masses attached to the atoms.

In a further exposition of the above theory, Dutt[2] has shown that the amount of strain is roughly proportional to the angular displacement of the valency directions

*Part I of these series of investigations was published in the Journal of the Chemical Society, London. The remaining parts were published in the Journal of the Indian Chemical Society. Full references are given at the end of the paper.

of the atoms and has the following values in the cases of double bonds occurring between carbon, oxygen and nitrogen atoms:

| | | |
|---|---|---|
| C=C | = | 219° |
| C=O | = | 289·5° |
| C=N | = | 229·5° |
| N=N | = | 240° |
| N=O | = | 300° |

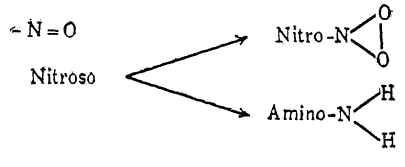
From the above exposition, it can be easily seen that the N=O or the "nitroso" group, should be the most fruitful cause of colour amongst organic compounds on account of the high degree of molecular strain contained therein, as calculated from theoretical considerations. This is the reason why most of the true nitroso compounds have very high absorptions as can be seen from table I:

TABLE I

Absorption maxima of nitroso compounds

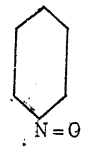
| Nitroso compound | Absorption maxima (Å) | Colour in alcoholic solution |
|---|---|---|
| Nitrosobenzene | 7300 | green |
| p-Nitrosotoluene | 7300 | green |
| Nitrosomesitylene | 7320 | dark green |
| Nitrosonaphthalene | 7350 | dark green |
| Ter-nitrosobutane | 6390 | dark blue |
| Ter-nitrosoisopropyl-acetone | 6600 | dark-greenish blue |
| Nitroso-antipyrine | 6650 | green |
| 3:5-dimethyl-4-nitrosopyrazole | 6250 | blue |

On account of the high strain in the molecule, the nitroso compounds are very easily reduced to the amino and oxidised to the nitro state by the weakest reducing and oxidising agents respectively, with the consequent loss of molecular strain and disappearance of intense colour:

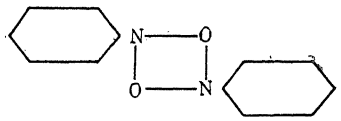



For the same reason also, most of the nitroso compounds tend to lose their internal strain by formation of more stable bi- or tri-molecular compounds. Thus the colourless and comparatively more stable nitrosobenzene in benzene solution is

found to be bimolecular, although in the vapour state it is intensely green, very unstable and monomolecular:

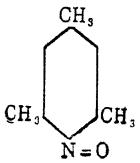


Nitrosobenzene
(Vapour, intense green)

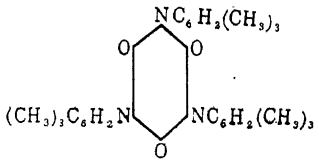


Nitrosobenzene
(Benzene solution, colourless)

Similarly, the unstable nitrosomesitylene in alcohol solution and also in the vapour state is emerald green and monomolecular, but in benzene solution and also in the solid state is colourless, stable and trimolecular:

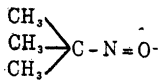


Nitrosomesitylene
(In alcohol, emerald green)

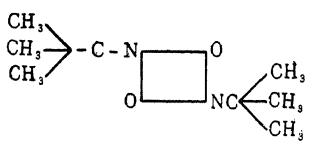


Nitrosomesitylene
(In benzene, colourless)

According to Bambarger,[8] who has studied the molecular weight of ter-nitroso-butane in solutions of organic solvents, the blue solutions are unimolecular, while the bimolecular solutions are colourless:

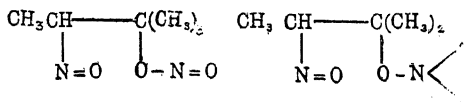


Ter-nitrosobutane
(In alcohol, blue)



Ter-nitrosobutane
(In benzene, colourless)

According to the findings of Wallach[9] and also of Schmidt,[10] the intense blue colour of alkylene-nitrosates and nitrosites like:



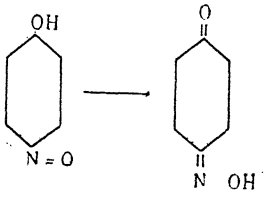
Nitrosite

Nitrosate

completely disappear and they become colourless by the formation of bimolecular compounds.

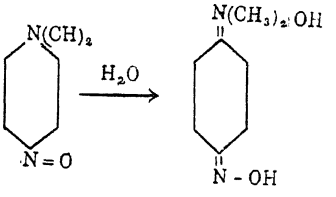
The internal strain of nitroso compounds is also lost by formation of isonitroso compounds whenever there is a possibility of isomerism by the migration of

a hydrogen atom in vicinity, as in the cases of nitrosophenols and nitrosonaphthols, which are generally only yellow in colour :



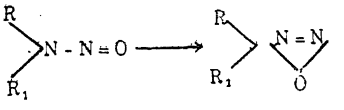
Nitrosophenol Quinoneoxime

Similar change also takes place by the action of water on nitroso-dialkyl-amines. Thus nitrosodimethylaniline is dark green in colour in the solid state, but in aqueous solution it is only yellow in colour :



(Green) Nitrosodimethylaniline (Yellow)

Aliphatic and aromatic nitroso-amines are light-coloured due to the loss of internal strain of the true nitroso group by rearrangement of valencies in which one of the nitrogen atoms becomes pentavalent:

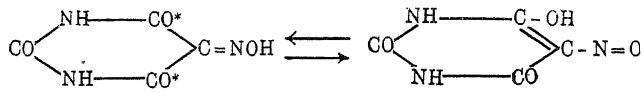


(Highly strained form) Nitrosoamine (Weakly strained form)

This is the reason why dimethyl-nitrosamine, phenylmethyl-nitrosamine, nitrosodiphenylamine, nitroso-carbazole, etc , are all yellow in colour. In fact there is no instance available in organic chemistry, where a nitroso group attached to a tertiary nitrogen atom ever exhibits any intense colour that is characteristic of a true nitroso group. It is only when a nitroso group is in such a position where it is quite incapable of tautomerism into a less strained configuration, that the true colour that is expected of the group from a consideration of its molecular structure is developed.

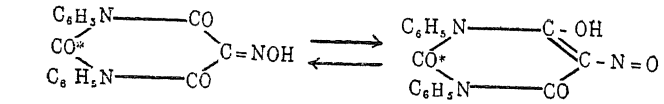
From a theoretical exposition of molecular strain as the product of distortion or angular displacement of the valency directions of atoms, it is quite easy to realise

that such a highly strained group as the nitroso (N=O) group will always tend to lose its high degree of molecular strain, by some means or other, as has already been pointed out, most usually by the formation of a weakly strained isonitroso group. But the reverse process of conversion of an isonitroso or oximino group into a nitroso group is not a phenomenon that is expected from theoretical considerations, but nevertheless is one that actually does occur in organic chemistry, although comparatively rarely and under special circumstances. In this way a weakly coloured isonitroso compound is transformed into as strongly coloured nitroso compound with simultaneous enhancement of molecular strain. A classical example of such a phenomenon is furnished by violuric acid as studied by Ghatak and Dutt,[11] in which the tautomerism between the two forms can be shown as given below :



Violuric acid (Oximino-ketonic form) ⟷ Violuric acid (Nitroso-enolic form)

On account of this we find that violuric acid is colourless in the solid state and also in solution in non-hydroxylic solvents and only pale pink in aqueous solution. But under special circumstances, that is by the influence of organic or inorganic bases, the tautomerism between the two forms is arrested and the comparatively more acidic nitrosoenolic form is stabilised by salt formation. This takes place particularly in view of the fact that the CO groups marked by asterisks are residues of carboxyl groups and retain their acidic character in a latent form which becomes dominant whenever there is a chance for enolisation by the migration of a hydrogen atom and the fixation of the enolic structure by salt formation. Thus we find that although violuric acid is colourless in the solid state and very pale pink in aqueous solution, yet its salts with inorganic and organic bases are all crimson in colour, the intensity of the crimson coloration being roughly proportional to the strength of the basic character of the base undergoing salt formation. This behaviour of violuric acid was found to be still more pronounced or intensified in the case of its symmetrical diphenyl derivative as studied by Prakash and Dutt,[12] who found that 1 : 3-diphenyl-violuric acid although colourless in the solid state and also in non-hydroxylic solvents, developed an intense violet colour on treatment with organic and inorganic bases due to salt formation, and consequent fixation of the nitroso-enolic structure.



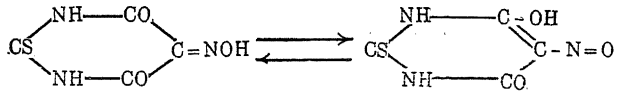
(Oximino-ketonic form) Colourless ⟷ 1 :3-diphenyl-violuric acid. ⟷ (Nitroso-enolic form) Violet salts.

The intensification of colour in the above case is apparently produced by loading of the violuric acid molecule by the two phenyl groups.

Still further intensification of colour of the above interesting compound was produced by additional loading, that is by substitution of the oxygen atom marked by an asterisk by a heavier sulphur atom, as in the case of 1 : 3-diphenylthiovioluric acid discovered and studied by Dass and Dutt.[13] These authors found that the latter substance although only orange-yellow in colour in the solid state and in solution in organic solvents, formed intense green-coloured salts on treatment with organic or inorganic bases, the colour change being sufficiently intense and rapid to make it an excellent indicator :

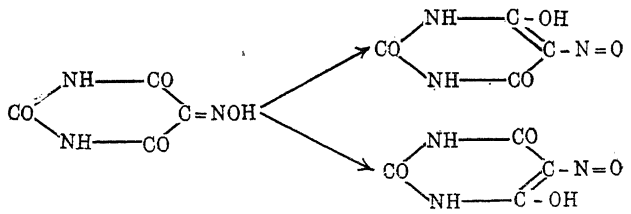
(Oximino-ketonic form) Orange-yellow — 1:3-diphenylthiovioluric acid — (Nitroso-enolic form) Green salts

The effect of loading on the intensification of colour by the above-mentioned method is also exemplified by the formation of violet salts from thiovioluric acid as studied by Lal and Dutt,[14] in place of pink salts obtained in the same way from violuric acid :



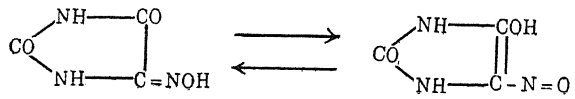
(Oximino-ketonic form) Pale yellow — Thiovioluric acid — (Nitroso-enolic form) Violet salts

In all the above instances, the tautomerism between the oximino-ketonic and nitroso-enolic forms is very rapid due to the possibility of two distinct nitroso-enolic structures in which the oximino-ketonic form may tautomerise, as can be easily seen in the following diagrams given for violuric acid :



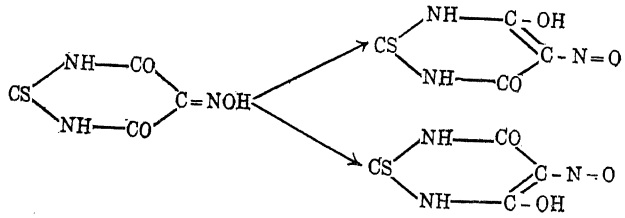
Consequently there is much greater possibility for the fixation of the nitroso-enolic structure by salt formation with its concomitant development of intense colour

in the above case, than in the case of isonitroso-hydantoin, where only one nitroso-enolic structure is capable of existence :

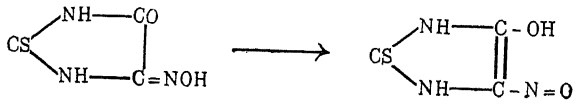


(Oximino-ketonic form) Pale cream — Isonitroso-hydantoin — (Nitroso-enolic form) Yellow salts

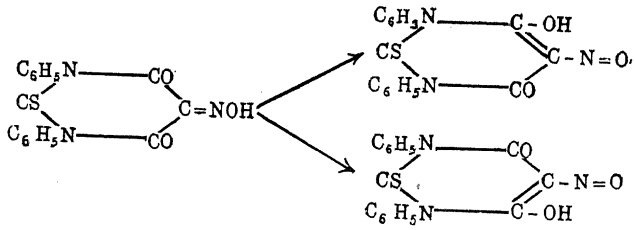
In the same manner, the behaviour of thiovioluric acid as studied by Lal and Dutt can be compared with the behaviour of isonitroso-thio-hydantoin as studied by the present author, and also that of 1 : 3-diphenyl-thiovioluric acid as studied by Dass and Dutt with the behaviour of 1 : 3-diphenyl-thiohydantoin studied by Dutt and Agarwal.[15] Whereas in the former cases there are two possibilities of nitroso-enolic structure formations, only one such exists in the latter cases. Consequently in the former cases the intensification of colour is much greater than in the latter. Thus :



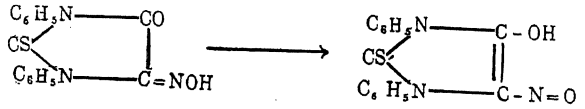
Thiovioluric acid (Purple salts)



Isonitroso-thiohydantoin (Yellow salts)

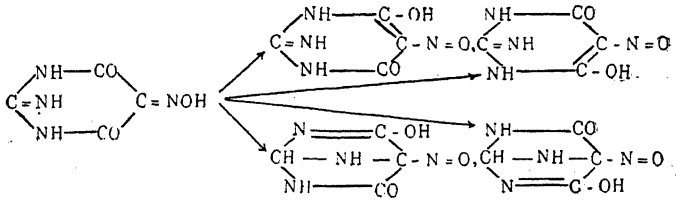


1 : 3-diphenylthiovioluric acid (Green salts)



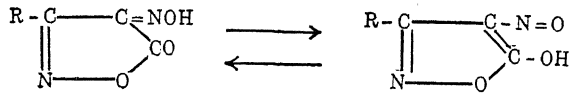
1 : 3-diphenylthiohydantoin (Crimson salts)

In the same way 1 : 3-diphenyl-violuric acid as studied by Prakash and Dutt and giving purple salts can be directly compared with isonitroso-diphenyl-hydantoin prepared by the present author and giving red salts. From the same point of view, even violuric acid having two nitroso-enolic forms and giving pink salts can be compared with isonitroso-malonyl-guanidine as studied by Dass and Dutt[16] having four nitroso-enolic forms and forming violet salts. In other respects, the two compounds are practically identical with each other:



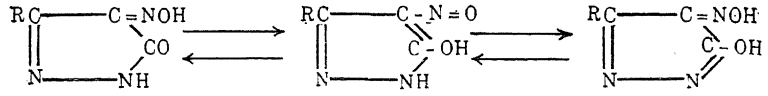
Isonitrosomalonyl-guanidine.

For the same reason, while 3-methyl-, and 3-phenyl-iso-oxazolones as studied by Dutt and Dass[17] develop crimson or deep magenta colours on treatment with alkali,



(Oximino-ketonic form) Isonitroso-iso-oxazolones (Nitroso-enolic form)

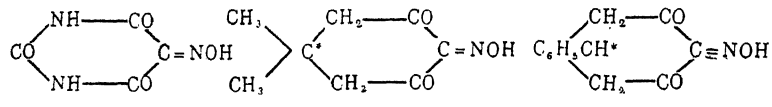
the corresponding isonitroso-pyrazolones are only yellow under identical conditions due to the fact that out of three possible tautomeric forms, only one contains the highly strained true nitroso group, which consequently has very little chance of existence in view of the highly acidic oximino-enolic form in which most of the salt formation occurs:



(Oximino-ketonic form) Isonitroso-pyrazolones (Nitroso-enolic form) (Oximino-enolic form)

The most interesting cases of colour phenomena with regard to isonitroso compounds are those of isonitroso-dimethylhydroresorcin and isonitroso-phenyl-hydroresorcin discovered by the present author, both of which yield intense blue-coloured salts with alkalis and organic bases. From the point of view of structure,

they have close resemblance to violuric acid, at least half the portion of the molecule of each being just the same:

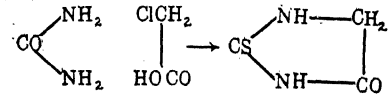


Violuric acid | Isonitroso-dimethyl-hydroresorcin | Isonitroso-phenyl-hydroresorcin

But whereas violuric acid forms pink salts with absorption maxima in the neighbourhood of 5400Å, isonitroso-dimethyl-, and isonitroso-phenyl-hydroresorcin form blue salts with absorption maxima in the neighbourhood of 6500Å. The increase in the intensity of colour in the latter two cases is mainly due to the application of load to the carbon atoms marked with asterisks, thereby affecting the distortion of the cyclohexane ring, as has already been pointed out by Thorpe and his co-workers[17, 18, 19, 20, 21]. Similar effect of increased load on this carbon atom has already been pointed out by Dass and Dutt,[13] who has shown that diphenyl-violuric acid forming violet salts is converted into diphenyl-thio-violuric acid yielding green salts by increase of load at this carbon atom by substitution of oxygen atom by sulphur.

## Experimental

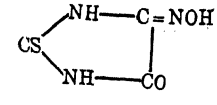
*Preparation of thiohydantoin.*—The so-called thiohydantoin was first prepared by Volhard[22] by the action of monochloroacetic acid on thiourea in alcoholic solution, the course of the reaction being assumed by the author to be as given below:



Thiourea | Monochloroacetic acid | Thiohydantoin (?)

(I)

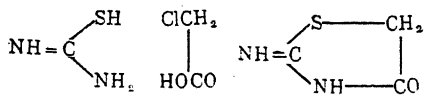
By the action of nitrous acid on this compound, Maly[23] obtained an isonitroso compound which consequently was supposed to have the following constitution:



Isonitrosothiohydantoin(?)

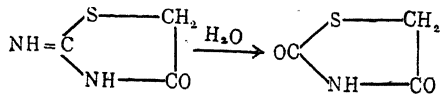
Subsequent research by a number of workers, particularly by Anreasch[24] has shown that by the action of monochloroacetic acid on thiourea, the product that is formed

is not thiohydantoin, but pseudo-thiohydantoin formed in accordance with the diagrams given below :



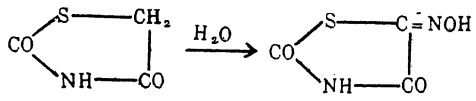
Thiourea Chloroacetic acid Pseudo-thiohydantoin (II)

This pseudo-thiohydantoin by the action of glacial acetic acid is converted into mustard-oil-acetic acid in the following manner as shown by Volhard[25] :



Pseudo-thiohydantoin Mustard-oil-acetic acid isonitroso

By the action of nitrous acid, this is then converted into isonitroso-mustard-oil-acetic acid with the following structure :



Mustard-oil-acetic acid Isonitroso-mustard-oil-acetic acid

This latter substance is a yellow compound dissolving in alkalis with a bright yellow colour, which was therefore mistaken by Maly[23] to be isonitroso-thiohydantoin. More recently Dass[26] has committed the same grievous mistake by taking isonitroso-mustard-oil-acetic acid to be isonitroso-thiohydantoin and preparing organic and inorganic salts out of it. Apparently the latter author must have been quite ignorant of the extensive work of Andreasch on pseudo-thiohydantoin.

Real thiohydantoin with the structure given above was prepared by the present author by a slight modification of the method of Dixon,[27] in the following manner: A mixture of dichloroacetic acid (13 gms.), thiourea (7·5 gms.) and water (100 c.c.) was heated under reflux on a sand-bath for two hours. The solution became cloudy due to separation of sulphur, which was filtered off. The clear liquid on basification with sodium carbonate, precipitated the thiohydantoin in colourless needles, melting at 176—78°C with decomposition. Yield was 6·2 grams.

The isonitroso compound was prepared in the usual manner by dissolving molecular proportions of thiohydantoin and sodium nitrite in dilute sodium hydroxide and pouring the cooled solution with vigorous stirring into a mixture of crushed ice and dilute hydrochloric acid. After keeping in the ice chest overnight, the light cream coloured precipitate was filtered off, washed with water, and recrystallised from a large quantity of boiling water in pale cream coloured needles melting above 220°C

with decomposition. The substance dissolves in alkalis and organic bases with a bright yellow colour. (Found S=22·52 ; theoretical S=22·27.)

*Preparation of isonitroso- 1: 3-diphenyl-hydantoin.*

(1) *1: 3-diphenyl-hydantoin.*—After trial of a number of methods given in literature particularly that by Bischoff and Hausdorfer[28] with unsatisfactory results, the following method was devised for the preparation of this substance in satisfactory yield and purity: A mixture of 10 grams of carbanilide and 6 grams of monochloroacetic acid with the addition of two drops of concentrated sulphuric acid was heated in an oil-bath at 150—160°C for about 3 hours with frequent stirring, until the evolution of hydrogen chloride was complete and the frothing subsided. The product was poured out while still fluid and on cooling was broken up and extracted with boiling water until chloracetanilide (M. P. 129-130°C) no longer crystallised out from the cooled extract. The insoluble residue was then crystallised from boiling alcohol, when 1 : 3-diphenylhydantoin crystallised out in glistening plates melting at 137°C. Yield obtained was 3·8 grams.

(2) *Isonitroso- 1 : 3-diphenyl-hydantoin.*

1 : 3-diphenylhydantoin (5 g.) was dissolved in warm (49°C) glacial acetic acid and treated with finely powdered sodium nitrite (10 g.), in small quantities at a time. The colourless solution became orange-red and on cooling and standing for some time, it deposited glistening yellow needles of the isonitroso derivative which were filtered off and washed with a little benzene. M. P. 128 °C with decomposition. The substance dissolves in alkalis and in solutions of organic bases with a red colour. (Found N=14·6; theoretical N=14·9 %.)

*Preparation of isonitroso-dimethylhydroresorcin.*—8 grams of pure twice recrystallised dimethylhydroresorcin (prepared according to the method of Kompa[29]) and 4 grams of dry sodium nitrite were dissolved in 50 c.c. of a 10 per cent solution of sodium hydroxide and the mixture thoroughly cooled in refrigerator. In a separate beaker, 20 c.c. of concentrated hydrochloric acid and 100 grams of crushed ice were vigorously stirred with a thermometer, and when the temperature had fallen to −10°C, the previously made and cooled solution of dimethylhydroresorcin added to it all at once. The mixture became orange-red and on continuing the stirring, very soon the isonitroso compound separated out as a glistening crystalline magma. This was stirred for another fifteen minutes, rapidly filtered at the pump and washed with a little ice-cold water. The substance was then dried over concentrated sulphuric acid in a vacuum desiccator kept inside the refrigerator. Light yellow glistening needles melting at 110°C with decomposition and gas evolution. The substance is slightly soluble in cold water, but easily soluble in hot and also in most of the organic solvents. The aqueous and alcoholic solutions on treatment with alkalis and organic bases develop intense indigo-blue colorations. In dry air the substance

is quite stable, but in presence of moisture and hot atmosphere, it very quickly undergoes decomposition, the nature of which is under investigation. (Found N = 8·4 ; theoretical N = 8·2 %.)

*Preparation of isonitroso-phenylhydroresorcin.*—9·4 grams of phenylhydroresorcin (prepared by a slight modification of the method described by Michael[30]) and 3·5 grams of dry sodium nitrite were dissolved in 150 c.c. of 2 per cent aqueous sodium hydroxide, and the solution cooled in a refrigerator. It was then gradually added with vigorous stirring to a mixture of 200 grams of crushed ice and 10 c.c. of concentrated hydrochloric acid which had already attained a temperature of —10°C. The isonitroso compound came down in the form of a light yellow crystalline precipitate. After standing for about 10 minutes, one gram of urea was added to the mixture to destroy any unreacted nitrous acid and the crystalline precipitate rapidly filtered and washed with ice-cold water. It was then dried in a vacuum desiccator over concentrated sulphuric acid inside a refrigerator. Light yellow prisms which have no definite melting point, but slowly undergo decomposition on heating. It is slightly soluble in cold water, more soluble in hot and easily soluble in organic solvents. Its aqueous and alcoholic solutions yield with alkalis and organic bases, intense indigo-blue colorations. (Found : N = 6·7 ; theoretical N = 6·4 %.)

TABLE II

Absorption maxima of some isonitroso-compounds and their salts.
Figures indicate wavelengths in Angstrom units.

| Name of the isonitroso compound | Absorption maxima of the | | | | |
|---|---|---|---|---|---|
| | Free acid (aqueous-alcoholic sol.) | Sodium salt | Potassium salt | Ammonium salt | Methylamine salt |
| Violuric acid ... ... | 5305 | 5830 | 5830 | 5832 | 5782 |
| Thiovioluric acid ... ... | 4403 | 5828 | 5847 | 5837 | 5892 |
| Isonitroso-malonylguanidine ... | 5860 | 5830 | 5835 | 5835 | 5850 |
| Diphenylvioluric acid .. ... | 5650 | 5835 | 5840 | 5845 | 5810 |
| Diphenylthiovioluric acid ... | 5130 | 6610 | 6560 | 6060 | 6010 |
| Isonitroso-hydantoin ... ... | 4250 | 4650 | 4700 | 4650 | 4620 |
| Isonitroso-thiohydantoin ... | 4100 | 4810 | 4850 | 4790 | 4790 |
| Isonitroso-diphenylhydantoin ... | 4430 | 5080 | 5150 | 5100 | 5090 |
| Isonitroso-diphenylthiohydantoin ... | 4435 | 5550 | 5600 | 5550 | 5090 |
| Isonitroso-3-methylisooxazolone ... | 5300 | 5860 | 5890 | 5770 | 5760 |
| Isonitroso-3-phenylisooxazolone ... | 5300 | 5850 | 5910 | 5810 | 5790 |
| Isonitroso-3-methylpyrazolone ... | 4170 | 5080 | 5180 | 4810 | 4932 |
| Isonitroso-3-phenylpyrazolone ... | 4650 | 4950 | 5045 | 4995 | 4928 |
| Isonitroso-dimethylhydroresorcin ... | 4450 | 6380 | 6410 | 6380 | 6380 |
| Isonitroso-phenylhydroresorcin ... | 4560 | 6550 | 6580 | 6550 | 6550 |

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# CHEMICAL EXAMINATION OF THE FATTY OIL FROM THE SEEDS OF *OCIMUM CANUM, SIMS*

By Ram Das Tiwari

Chemistry Department, University of Allahabad

Communicated by Dr. S. Ghosh

(Received on December 21, 1940)

SUMMARY

The oil from the seeds of Ocimum canum, sims, has been examined in detail. The presence of the following has been established and their percentages determined ;—

| | |
|---|---|
| Linolenic acid. | 5·44 |
| Linolic acid. | 4·18 |
| Oleic acid. | 58·70 |
| Palmitic acid. | 19·11 |
| Stearic acid | 2·87 |
| Arachidic acid. | 1·69 |
| Unsaponifiable matter (sterol). | 0·48 |


*Ocimum Canum, Sims* popularly known as Mamri or Ramtulsi in Hindustani is a strongly scented low shrub belonging to the Labiate family. During rainy season it grows abundantly on the river banks and moist places throughout the United Provinces and foot hills of Kamaon and Nepal. The plants are erect, much branched and pubescent between 1 and 2 feet in height. The description of the plant is given in Kirtikar and Basu.[1]

The plant is highly medicinal. During[2] fever when the extremities are cold, the leaves made into a paste are applied to fingers and toe-nails; the same preparation is also used as a cure for parasitical diseases of the skin. Its use in skin diseases has also been mentioned by Chopra.[3] A decoction of the leaves of the plant is beneficial in diseases of digestive system and stomach troubles.

In spite of its great medicinal value very little work has been done on the chemical examination of this plant; only the essential oil has been examined. (L. S. Glichitch and Y. R. Naves, Chim. et Ind. 1933, **29**, Sp. number 1029—33; Annon, Bull. Imp. Inst. 1934, **32**, 195—252; A. Rotermel Pharm. Ztg. 1935, **80**, 337—340; Schimmel & Co., Annual Rept. 1936, 7-8, 26, 76, 87-88.)

Tayal and Dutt[4] examined the essential oil from the plant and established the presence of linalool, geraniol, citronellol, citronellal and methyl heptenone but they did not make any reference of the work quoted above.

In the present investigation the fatty oil of the seeds of the plants popularly known as *Tukhami rehan* has been examined. The seeds are small, spherical and black and give a mucilage when soaked in water.

## Experimental

Five seers of seeds obtained from the local market* were crushed and exhaustively extracted with benzene in a 5 litre extraction flask. On distilling off the benzene, a dark coloured oil was obtained which was purified by animal charcoal and fuller's earth giving a transparent oil. The seeds were found to give 11·6% of the oil. The oil was a semi-drying one, having the following physical and chemical constants. The oil deposited a small sediment on keeping, the amount of which was too small to be investigated.

Table 1

| | |
|---|---|
| Specific gravity at 26°c | 0·9102 |
| Ref. Index at 25°c | 1·4715 |
| Acid value | 8·6 |
| Saponification value | 189·1 |
| Acetyl value | nil |
| Unsaponifiable matter | 0·48% |
| Hehner number | 93·3 |
| Iodine value | 93·8 |

300 gms. of the oil were then saponified in the usual manner with alcoholic potash and the soap so formed was dried. It was then exhaustively extracted with ether and the unsaponifiable matter was removed. The soap remaining after the removal of the unsaponifiable matter was decomposed with dil. sulphuric acid in presence of petroleum ether and the fatty acids were obtained in solution in petroleum ether. The petroleum ether extract was washed with water, dehydrated with sodium sulphate and on removal of the solvent, fatty acids were obtained having the following physical and chemical constants:—

Table 2

| | |
|---|---|
| Consistancy | liquid |
| Sp. gr. at 25°c | 0·9172 |
| Ref. Index at 28°c | 1·4126 |
| Neutralisation value | 202·8 |
| Mean molecular weight | 277·2 |
| Iodine value | 80·7 |

*The authenticity of the seeds was tested by actually sowing these seeds and identifying the plant with the help of Mr. G. D. Srivastava of Botany Department of the Allahabad University.

The mixture of the fatty acids was then separated into saturated and unsaturated acids by Twitchell's Lead-salt alcohol process.[5] The following table gives the percentage, iodine value, neutralisation value and the mean molecular weight of the solid and liquid acids:—

TABLE 3

| Acid | Percentage | Iodine value | Neutralisation value | Mean mol. wt. |
|---|---|---|---|---|
| Solid | 26·8 | 5·73 | 212·2 | 264·34 |
| Liquid | 73·2 | 107·97 | 198·8 | 281·49 |

EXAMINATION OF THE UNSATURATED ACIDS

The constituents of the unsaturated acids were determined by method originally suggested by Eibner and Muggenthaler[6] and later on worked up extensively by Jamieson and Boughmann[7]. According to this method the bromine addition products of unsaturated acids were prepared and separated as follows:—

To a known weight of the mixture of acids dissolved in dry ether and cooled to −10°c bromine was added till it was in slight excess and the temperature was not allowed to rise above −5°c during this process. The mixture was then allowed to stand for 2 hours at −10°c. The hexabromide of Linolenic acid being insoluble in ether was precipitated. The precipitate was filtered, washed with dry ether and weighed. The melting point of the hexabromide was found to be 175·5°c (Theo. 177°c). The weight of Linolenic acid was then calculated from the weight of the hexabromide.

The ethereal solution consisting of filtrates and washing was treated with an aqueous solution of sodiumthiosulphate in a separating funnel and the excess bromine was thus removed. The ethereal solution was washed, dried with anhydrous sodium sulphate and ether removed by distillation. The residue was taken up with about 250 c.c. of dry petroleum ether, boiled in order to make a solution and kept overnight in a frigidare. On standing the tetrabromo-linolic acid was precipitated. It was filtered and washed with dry petroleum ether. The filtrate and washings were concentrated to about 50 c.c. cooled and again allowed to stand overnight in the frigidare The second crop of the tetrabromide so obtained was added to the first and weighed. It was found to melt at 115°c (Theo. 114°c). Finally the petroleum ether filtrate was evaporated to dryness and weighed. The bromine contents of the residue were determined by Piria and Schiff's method, and from this the percentages of dibromide of Oleic acid and tetrabromide of Linolic acid were calculated and hence the weights of the two acids were found (table 4).

| | |
|---|---|
| Wt. of Unsaturated acids | 76·5 gms. |
| Wt. of Hexabromide | 16·5 gms. |

| | |
|---|---|
| Wt. of Linolenic acid | 6·056 gms. |
| Percentage „ „ | 7·97 gms. |
| Wt of Tetrabromide | 19.71 gms. |
| Wt. of Linolic acid | 0·904 gms. |
| Wt. of Dibromide + tetrabromide | 110·6 gms. |
| Percentage of Bromine | 37·4 gms. |
| Wt. of Tetrabromide | 7·866 gms. |
| Wt. of Dibromide | 102·734 gms. |
| Wt. of Linolic acid in the residue | 3·671 gms. |
| Wt. of Oleic acid | 65·55 gms. |
| Total wt. of Linolic acid | 4·575 gms. |

TABLE 4

| Acid. | Weight in gms. | Percentage in liq. acids. | Percentage in mixed acids. |
|---|---|---|---|
| Linolenic | 6·056 | 7·97 | 5·83 |
| Linolic | 4·575 | 6·11 | 4·48 |
| Oleic | 65·55 | 85·79 | 62·89 |

The percentage of these acids in the liquid acids were also calculated from the knowledge of mean molecular weight and Iodine values of these acids and also of the liquid acids. The following table gives the result for comparison :—

TABLE 5

| Acid. | As found by Bromine addition. | On the basis of calculation. |
|---|---|---|
| Linolenic | 7·97 | 8·01 |
| Linolic | 6·11 | 6·2 |
| Oleic | 85·79 | 85·81 |

## EXAMINATION OF THE SATURATED ACIDS

The solid acids were converted into their methyl esters : the acids were dissolved in absolute methyl alcohol and a current of dry hydrochloric acid gas was passed in till the solution was saturated. The mixture was heated under reflux on a water-bath for about 15 hours. The esterified product was neutralised with sodium bicarbonate and distilled water was added to it. The ester layer at the top was separated and the aqueous layer was repeatedly extracted with ether. The ethereal solution was washed with water, dehydrated by anhydrous sodium sulphate and the solvent removed by distillation. They were then subjected to fractional distillation under reduced pressure. The boiling point and pressure were recorded in each case and the weight of each fraction determined. The neutralisation value, the

mean molecular weight and the iodine value were also determined (table No. 7). The acids from each fraction were isolated by fractional crystallisation and their melting points determined and were found to be Palmitic acid (M. pt. 62°c), Stearic acid (M. pt. 68°c) and Arachidic acid (M. pt 76°c). The following table contains the results of fractional distillation :—

TABLE 6

Weight of the ester taken 10 gms.

| Fraction No. | Boiling range. | Pressure. | Weight of ester in gms. |
|---|---|---|---|
| 1 | 170°—175°c | 10 mm. | 7·35 |
| 2 | 175°—180°c | 10 mm. | 0·93 |
| 3 | 180°—185°c | 10 mm. | 1·16 |

TABLE 7

| Fraction No. | Iodine value of ester. | Neut. value of ester. | Mean mol. wt. of ester. | Palmitic acid. | | Stearic acid. | | Arachidic acid. | | Unsaturated acids. | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | gm. | % | gm. | % | gm. | % | gm. | % |
| 1 | 3·4 | 205·9 | 273·3 | 6·6 | 90·7 | 0·12 | 1·8 | ... | ... | 0·17 | 2·42 |
| 2 | 21·8 | 193·5 | 290·5 | 0·18 | 20·0 | 0·55 | 59·2 | ... | ... | 0·14 | 15·36 |
| 3 | 19·7 | 178·6 | 314·1 | ... | ... | 0·35 | 30·1 | ·60 | 51·1 | 0·16 | 14·2 |
| | | | | 6·78 | | 1·02 | | 0·6 | | 0·47 | |

Total ... 8·87 gms.

The percentage of various saturated acids in solid acids and mixed acids are given in table 8:—

TABLE 8

| Acid. | Percentage in solid acids. | Percentage in mixed acids. |
|---|---|---|
| Palmitic | 76·43 | 20·48 |
| Stearic | 11·50 | 3·08 |
| Arachidic | 6·77 | 1·81 |
| Unsaturated | 5·31 | ... |

The following table gives the composition of fatty acids and their percentages in the oil :—

TABLE 9

| Acid | Percentage in acids. | Percentage in oil. |
|---|---|---|
| Linolenic | 5·83 | 5·44 |
| Linolic | 4·48 | 4·18 |
| Oleic | 62·89 | 58·7 |
| Palmitic | 20·48 | 19·11 |
| Stearic | 3·08 | 2·87 |
| Arachidic | 1·81 | 1·69 |
| | Total =98·57 | |

The remaining 1·43 per cent were the unsaturated acids that came along with the saturated ones.

*Unsaponifiable matter*:—The unsaponifiable matter obtained on removal of the ether was crystallised from alcohol whereby colourless crystals m. pt 134°c were obtained. It was found to be an unsaturated compound giving reactions of a sterol. The amount being too small it could not be examined.

ACKNOWLEDGEMENTS

The author is very grateful to Dr. S. Dutt for his generous guidance and supervision throughout this investigation, to Dr. B. K. Singh, Professor of Chemistry, University of Allahabad, for his valuable help in going through the manuscript, and to Mr. Brij Mohan Saran Agarwal, Kanta Prasad Research Scholar, for his help in writing the paper.

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# A BRIEF NOTE ON THE "CAMEL-SPIDERS" (*GALEODES*) OF THE HYDERABAD STATE

By M. Rahimullah

Zoology Department, Osmania University, Hyderabad (Deccan)

Communnicated by Prof. D. R. Bhattacharya

(Received on July 18, 1940)

SUMMARY

One species of *Galeodes*, viz., *G. indicus*, Pocock, from Hyderabad (Deccan) has been described in this paper. The differences between ♂ and ♀ and their specific characters have been given. Their habits have been described along with some other interesting points.

Pocock has described four species of *Galeodes* from India in the *Journ. Bombay Nat. Hist. Soc.*, Vol. IX, 1895, and the same author has also mentioned seventeen species of these arachnids in the Fauna of British India, 1900. Besides these two valuable accounts there is no systematic description of the *Galeodes* of India, and only one species has been casually mentioned from Secunderabad* (Ricardo, type) by Pocock in the Fauna of Brit. India.....the species described is *Galeodes indicus*, sub-species *australis*, nov. (*Orientalis*, Simon).

I have been making a series of observations and collecting *Galeodes* from various parts of Hyderabad, and during my search I was able to collect about 80 specimens. I observed that they are, as a rule, nocturnal in habit, and keep themselves hidden during the day under stones or in holes, coming out only at night time in quest of food, which consists mainly of insects—cockroaches form a favourite item on their menu; and I believe that they come inside the houses particularly in search of these insects. They are found in plenty shortly before the rainy season, *i.e.*, in the months of April and May, and usually come near the light of a Petromax lamp or an ordinary lantern. I suppose they are not attracted by the light itself, but by the insects which hover round the light and form the food of these arachnids.

In my collection I got both males and females, but the number of males was much in excess to those of the females.....this is evidently due to most of the specimens being captured in April and May, which represent the breeding season when a vast number of them come out for mating, as several young ones have been caught subsequently in June and July.

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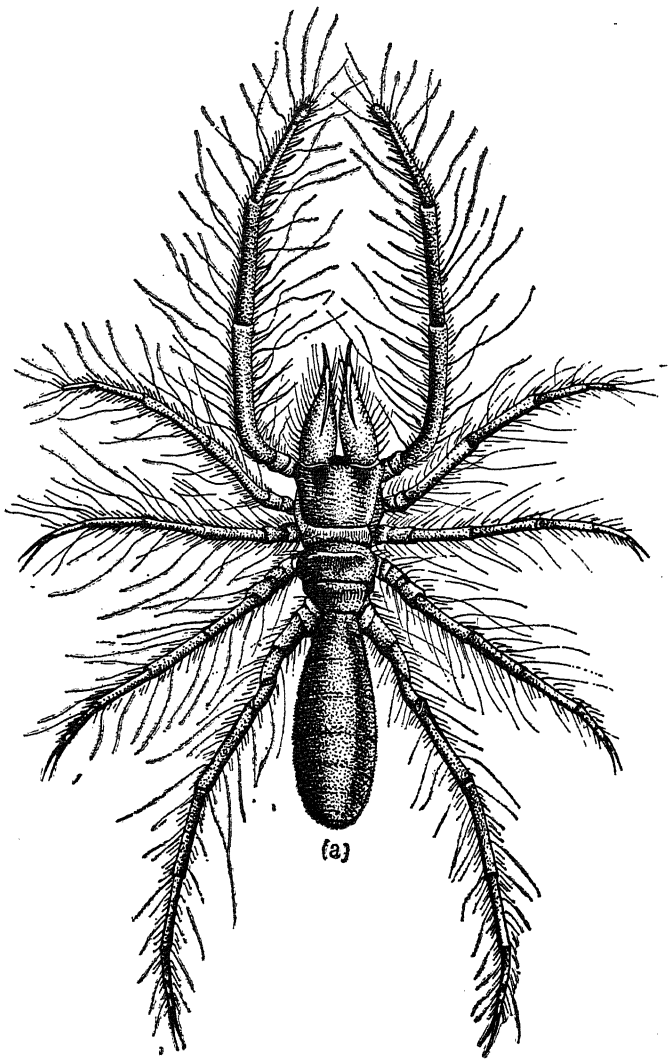
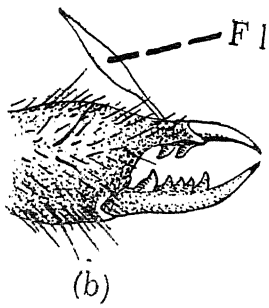
* It is a military Cantonment town within the Hyderabad State.

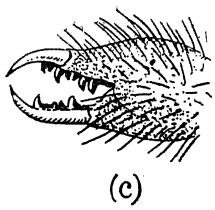
All my specimens belong to only one species, *viz*, *Galeodes indicus*, Pocock, and amongst these four fall under the sub-species *obscurior* (Pocock), (Text fig.).




Dorsal aspect of the ♂ *Galeodes indicus*, Pocock (x 2).




Lateral view of the jaw of the ♂ *Galeodes indicus*, Pocock, showing flagellum (Fl.) (x 2).




Lateral view of the jaw of the ♀ *Galeodes indicus*, Pocock (x 2).

*Galeodes* belongs to the Order *Solifugae* of the class *Arachnida*. They are locally known as "Jalmandal", and usually considered to be very poisonous by the people of Hyderabad. It is erroneously believed that their bite is fatal, and there is no remedy for it. This is indeed a very wrong notion, because these arachnids are non-poisonous, as has already been stated by Warburton in his account of these creatures in the *Cambridge Nat. Hist.* series 'Crustacea and Arachnida', 1923 as follows: "Several investigators have allowed themselves to be bitten without any special result. Some zoologists have found and described what they have taken to be poison-glands, but these appear to be the coxal glands, which have an excretory function." "The cutting powers of the immensely-developed chelicerae are usually sufficient to ensure a fatal result on small animals without the agency of poison. Distant, indeed, thinks they cannot be poisonous, for when birds attack them they flee before their assailants." (*cf. Camb. Nat. Hist.*, p. 424).

In this connection I may cite here an observation made by one of my friends, Mr. Joseph Fernandez, Museum Curator of this department, on a galeodes which was running on the wall of his room. A spider seeing it at once jumped over it and began to spin its web round the little animal. The galeodes neither attacked the spider in self-defence, nor did it try to run away from it out of fear. When it was ensnared in the meshes, Mr. Fernandez was able to secure the spider as well as the galeodes. If the galeodes were poisonous, it would have certainly attacked the spider at least in self-defence, and the spider would never have ventured to capture a creature endowed with poison.

Regan, however, in his '*Natural History*' says (p. 76), "They are predaceous, and feed on insects, lizards and mice. Some of them (*e.g.*, *Galeodes*) are large and formidable looking creatures, and some of them are venomous."

It would be interesting also to quote here a few lines from Pycraft's *Natural History* (pp. 398-399), "Most Solifugae are nocturnal, but a few are lovers of sunshine. They are extremely rapid in their movements, so that they are difficult to catch, and hold the first pair of legs before them as organs of touch. It is generally believed that they are venomous, but, as a matter of fact, they produce no poison. The strength of their chelicerae is sufficient to produce very serious wounds in the bodies of the insects which form their prey. They also fight with each other with extreme ferocity."

Now, I wish to divert the attention of my readers for a while to another interesting phenomenon, *viz.*, 'Why are the arachnids so much dreaded?' It is not actually due to the poison, but because of the agility with which these animals run. I may here cite a few lines regarding this point from an account of Arachnida by Theodore H. Savory as follows:

"The sudden running of a spider across the floor, of a scorpion across the tent cloth, of a solifuge across the desert sand attracts the eye, and as the attempt is

automatically made to "keep the eye on" the moving animal, images of the background pass across the retinae. The natural response follows and, since all motion is relative, the conscious mind has no difficulty in projecting the origin of the fear to the moving animal."

"In complete proof of this explanation of the fear induced by the Arachnida, there is the fact that the sensation appears only in response to a certain speed, and slowly moving images do not evoke it. Thus the moving animal to which the conscious mind transfers its emotions must itself run rapidly. Here is the explanation of the fact that fear of rapid animals is common all over the world. Actually it is not the scorpion or the spider which in the first place is responsible for our terror, but the rapidity of its motion. No one is afraid of a tortoise or of a snail."

I am much indebted to Professor D. R Bhattacharya for his kindly communicating this paper for publication. I also wish to thank Dr. F. H. Gravely, Superintendent of the Government Museum, Madras, for his kind help in the identification of these specimens, and to Professor B. K. Das of this department for his kind advice and valuable suggestions. My thanks are also due to Mr. M. Saidul Haq for preparing the illustrations.

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# ON THE STRUCTURE OF THE SO-CALLED PYLORIC CAECA IN A MARINE GENUS *PLATYCEPHALUS**

By M. Rahimullah

Zoology Department, Osmania University, Hyderabad (Deccan)

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SUMMARY

In this paper the author has described the structure of the so-called pyloric cæca in two species of marine fishes, belonging to the genus *Platycephalus*. The number of cæca in one species, *viz*. in *P. scaber* is 3-4 in the right and 3 in the left group, while in the other species, *viz*., *P. insidiator* the number of cæca is 9, disposed off in two groups. The intestine has got two loops in each species. Blood-and Nerve-supplies have also been traced.

## Introductory

Day, in his account of "Fishes", 'Fauna Brit. Ind.', vols. I & II (1889), has casually mentioned the presence of pyloric cæca in some Indian fishes. According to him these structures are present in about 31 families, including nearly 76 genera and about three times as many species. I have already given an account of the so-called pyloric cæca in three of the Indian fresh-water fish families, *viz*., Ophiocephalidae (1934), Notopteridae (1935) and Rhynchobdellidae (1936) in the Proc. Ind. Sc. Cong. A paper of mine on the cæca of Ophiocephalidae has also been published in 'Anat. Anz.', vol. 80, 1935.

After consulting the relevant literature on this subject, one would find that the structure of the pyloric cæca of other fishes has been described by Rosenthal (1824). Hyrtl (1864), Blanchard (1882), Stirling (1884), Fr. Day (1887), Bonduoy (1897 and 1899), Johnson (1907), Kostanecki (1913) and a few others.

## Historical Summary

In 1824 Rosenthal just touched upon the condition of the Pyloric appendages in the swordfish without giving any figures. Hyrtl (1864) has shown the curious mode of opening of the bile-duct into "appendices pyloricae" in *Fistularia*, *Aulostoma* and *Acanthurus*. Johnson (1907) has published a paper on "the individuality and variation of the pyloric cæca of the Centrarchidæ". The main object of his contribution is to show that the pyloric cæca of certain members of this family, *viz*., the Sun-fish, *Lepomis*, Black Bass, *Micropterus*, etc., are not similar in number and

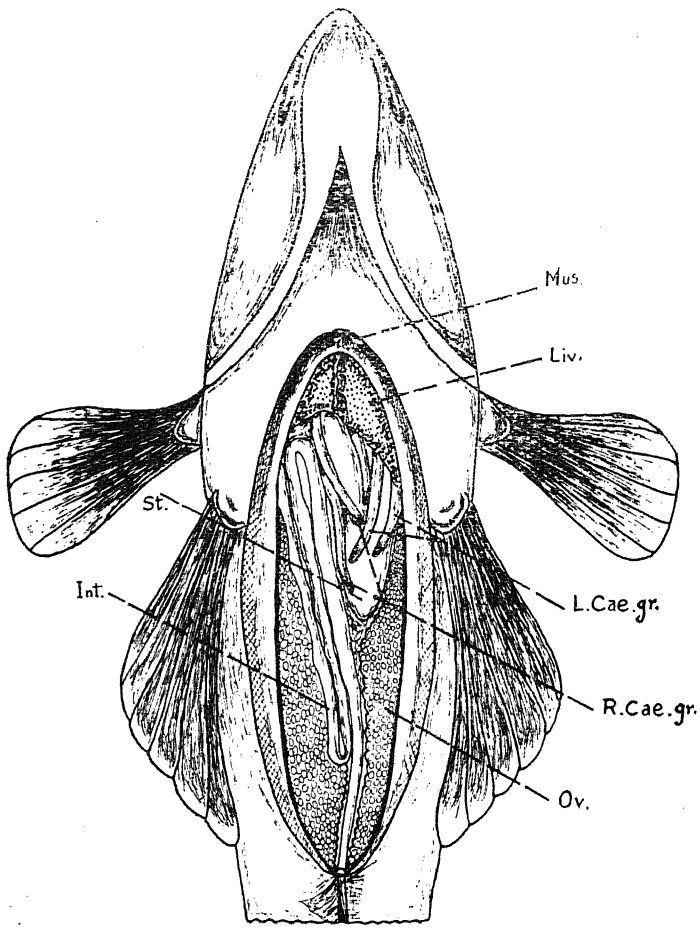
* A short summary of this paper was read by the author before the Zoology Section at the 24th annual meeting of the Ind. Sc. Congress held at Hyderabad (Deccan) in 1937.

form; in other words, they show variations; *i.e.*, to say, they have an individuality of their own.

THE SO-CALLED PYLORIC CAECA IN THE GENUS *Platycephalus*.

(A) *Topography & Morphology:*

Two species of this genus, *viz.*, *Platycephalus scaber* (Linn.) and *P. insidiator* (Forsk.) obtained from Ennur (Madras Presidency) were examined. It was noted that the number of caeca varies from 6-7 in *P. scaber*: they are arranged in two groups, right and left (Text-fig. *a* and *b*, R. cae. gr., L. cae. gr.), usually consisting of




(*a*) X2—Dissection of the viscera from the ventral aspect of *Platycephalus scaber* showing the caeca *in situ*.

*Ant. lp.*, Anterior loop of the intestine; *Bl. dc.*, Bile-duct; *Int.* Intestine; *L. Cae. gr.*, Left group of the so-called pyloric caeca; *Liv.*, Liver; *Mus.*, Muscles; *Oes.*, Oesophagus; *Ov.*, Ovary; *Pest. lp.*, Posterior loop of the intestine; *Pyl.*, Pylorus; *R. Cae. gr.* Right group of the so-called pyloric caeca; *St.*, Stomach.

3 or 4 in each group and lying on the ventral side of the stomach (st.). Usually the cæca are hidden from view by an envelope of fatty tissue on their ventral surface; and when this mass of fat is removed, the distal portions of all the three cæca of the *left* side are visible, only their proximal portions are covered over by the liver (liv.), whereas only two cæca of the right side are exposed, and the remaining one of this side is concealed from view due to the encroachment of the intestinal loop. In *P. insidiator*, on the other hand, there are nine cæca which are arranged in two groups, of 5 and 4 in each, and they are disposed practically in the same manner as in the other species

The ratios of the average length of the smallest and the largest cæca to the whole length of the alimentary canal are as follows:

(*i*) *In Platycephalus scaber:*

The smallest cæcum: alimentary canal = 1 : 9·4

& the largest cæcum: alimentary canal = 1 : 4·6

(*ii*) *In Platycephalus insidiator:*

The smallest cæcum: alimentary canal = 1 : 14·3

& the largest cæcum: alimentary canal = 1 : 10·2

These figures thus show that in *P. insidiator* the cæca are relatively much smaller than those of *P. scaber* as compared with the size of the alimentary canal.

(B) *Blood-and Nerve-Supplies:*

(*a*) The coeliaco-mesenteric artery gives off three branches:

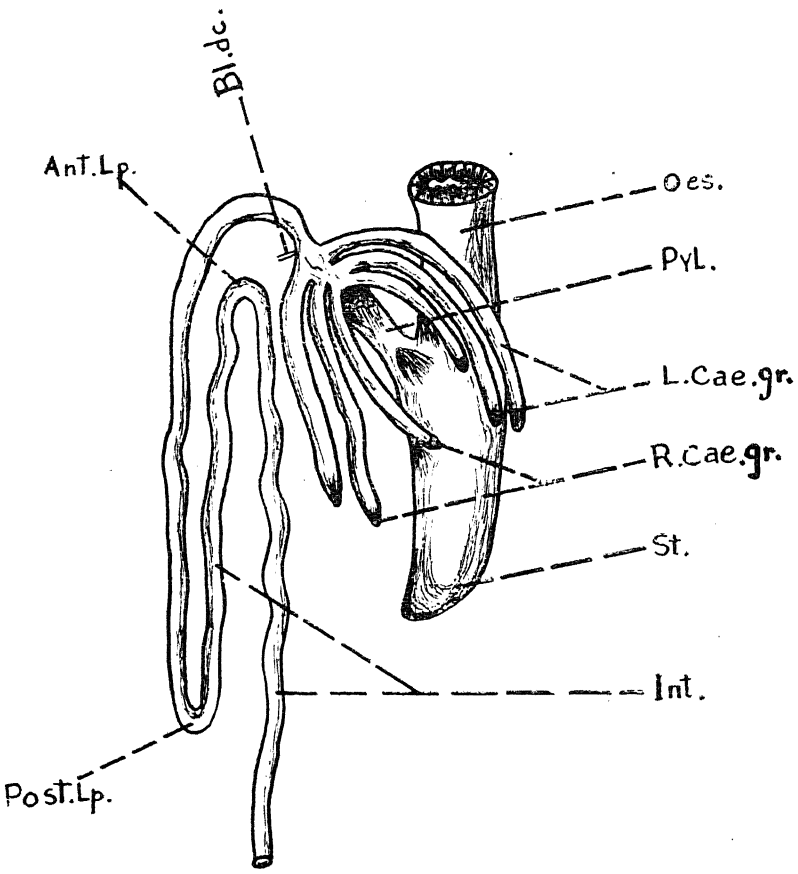
(*i*) Gastric—supplying the stomach.

(*ii*) Intestinal—supplying the intestine.

(*iii*) Cæcal—divides into two smaller vessels, right and left, and from each of these two vessels much smaller branches are given off which supply blood to all the cæca of the respective sides.

(*b*) The blood is returned from all the cæca of the right and the left sides by the small right and the left cæcal veins independently into the Hepatic Portal vein.

As regards the nerve-supply it may be said that the right visceral branch of the Vagus sends off small branches to both the right and left groups of the cæca, whereas its left counterpart innervates the stomach and the mesentery.




(*b*) X2—Alimentary canal of the same fish unravelled, showing the disposition of the caeca.

(Lettering as in Fig. *a*)

(C) *Histology:*

With regard to the histology of the so-called pyloric cæca in *Platycephalus* the following salient points are worthy of note, and may be briefly stated as follows:

(1) The serous membrane and other layers of the cæcal wall are present, and are almost similar in character to those found in other fishes previously described by me, with this difference that the muscle layers and the submucosa are comparatively reduced in thickness in *Platycephalus*. In all essential points the microscopic structure of the cæcum is similar to that of the proximal part of the small intenstine.

(2) The mucosa is thrown into a very large number of finger-like so-called cæcal villi (as in Ophiocephalidæ) which are more numerous as compared with other families already investigated by me.

(3) The so-called cæcal villi proliferate, unite and fuse with one another . . . . . the union and fusion of the mucous folds are being very strongly marked towards the distal half of each cæcum, extending right up to its very tip, and thus forming a copious mass of loose, so-called "spongy tissue" which obliterates practically the whole of the lumen of that region of the cæcum.

The wide extension of the so-called "spongy tissue" (*i.e.*, of the mucous folds) inside the cæcal lumen evidently serves to increase the area of the absorptive surface, and is a very characteristic feature of *Platycephalus* cæca, and is probably correlated with their relatively much smaller size as compared with that of the cæca in other groups of fishes, which are proportionately much bigger, and, consequently, bulk for bulk, the amount of the so-called "spongy tissue" is much less developed in the latter. The larger size of the so-called pyloric cæca of other fishes with the relatively small amount of the so-called "spongy tissue" present in them seem to me in a way to compensate for the smaller size of the pyloric cæca, the prolificity in number of the so-called cæcal villi and the magnitude of the absorbing tissue (*i.e.*, the so-called "spongy tissue") found in *Platycephalus*.

(D) *Physiology :*

In this connection the works of the following authors are important and worth mentioning, *viz.*, of Blanchard (1882), Mordacai (1882), Stirling (1884), Bonduoy (1899) and Pixell (1908). It has been stated that the cæca produce diastatic and trypsin like enzymes, which effect some digestion of the carbohydrates and proteids, and thus help the digestive processes of other juices poured into the alimentary canal.

The nature of the semi-digested food in the cæca, the opening of the latter into the ileum, the copious blood-supply to these structures, the drainage of the blood from them into the portal system and the structure of those digitiform so-called cæcal villi, indicate that the following physiological functions might probably be attributed to them :

(*a*) The cæca probably serve as accessory food-reservoirs . . . . the intestine in the fishes investigated is relatively short, but this cannot be assumed in all cases, until a large number of fishes has been thoroughly examined.

(*b*) In the cæca probably food is partly digested.

(*c*) In the cæca some absorption of the digested food also possibly takes place.

It is not yet certain if diet has any particular influence on the relative size and structure of "pyloric cæca", and this can be elucidated only by further study.

## Summary and General Conclusions

(1) The so-called pyloric cæca are outgrowths of the intestine as has been wholly corroborated by their histological structure, and hence the name pyloric cæca, as has already been pointed out by previous workers, is a misnomer.

(2) They are not to be mixed up with the cæcal (or rectal) gland of Selachian fishes which has been so thoroughly worked out by Crofts in recent years (cf. P.Z.S., 1925), and are not homologous either with this gland or with the "vermiform appendix", or with any other cæcal outgrowths of other vertebrates, because all such latter structures take their origin between the large and small intestine, whereas the so-called pyloric cæca are given off immediately behind the pylorus as true outgrowths of the first part of the ileum. No lymphoid tissue has been found associated with them which is characteristic of the cæcal gland of Selachians.

(3) Phylogenetically (and as a hypothetical case) the simplest and the most primitive type of the so-called pyloric cæcum may probably be represented by a glandular thickening of the mucous membrane, or as a very simple protuberance or outgrowth of the anterior part of the ileum. From such a most simple condition, by gradual evolution, the single cæcum of *Fistularia villosa* (Fam. Aulostomatidæ) might have arisen, and gradually the number of the cæca might increase, one after the other (either due to new developments, or by branching), and at the same time they may also attain complexity of structure, culminating in such a case as the Sturgeon in which all such outgrowths unite together giving rise to a compact glandular body with a single efferent duct, or the duct may be multiple as in a few other fishes.

Here I must express my sincerest thanks to Dr. B. Sundra Raj, for all facilities afforded me in the laboratory at Ennur. I am very grateful to Professor D. R. Bhattacharya for his kindly communicating this paper for publication in this journal. My thanks are also due to Dr. B. K. Das for his constant help and guidance in the preparation of this paper.

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# SOME OBSERVATIONS ON THE CYTOLOGY OF *SAPROLEGNIA DELICA* COKER

By R. K. Saksena and K. S. Bhargava

Department of Botany, University of Allahabad

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## SUMMARY

1. An account of the cytological studies of the vegetative mycelium of *Saprolegnia delica* Coker is given.

2. Observations on the living material were made both with and without vital staining. When no stain was used, fat particles, filamentous mitochondria, vacuoles, nuclei and pre-existing corpuscles were observed in the hyphae. With the help of vacuolar dyes the origin and evolution of the vacuolar system was studied. Of all the dyes neutral red proved to be the least toxic. Sphaerocrystals became visible with neutral red. No metachromatic corpuscles were found in the hyphae. With janus green Höcht B and dahlia violet, filamentous mitochondria became more distinct and were also seen in the process of fragmentation and vesiculisation. Janus green, which was less toxic than dahlia violet, formed a reduction product.

3. The mycelium was fixed in a number of nuclear and mitochondrial fixatives. For the fixation of mitochondria Helly's liquid and corrosive sublimate-formol and for the nuclei Flemming's weak solution modified by Saksena proved most satisfactory. In fixed preparations the structure of the mitochondria was similar to that observed in the living condition. The main portion of the nucleus was seen to consist of a central body which takes a dark stain with iron-alum haematoxylin. Surrounding this was a layer of nucleo-hyaloplasm, which was bounded externally by a distinct nuclear membrane. No mitotic division was observed.

## Introduction

Of the various genera belonging to the family Saprolegniaceae the genus *Saprolegnia* has received considerable attention from a number of workers from the point of view of its cytology, reproduction and physiology. Dangeard[2] and Guilliermond[4] were the first to study the mitochondrial and vacuolar systems in the vegetative hyphae of this genus. Joyet-Lavergne[10] demonstrated the oxido-reduction power of its mitochondria, while Milovidov[11, 12] studied their chemical constitution and the influence of various external factors on them. Famin[3] utilised this fungus to show the action of heat on its cytoplasm and mitochondria. Smith[15] in his studies of this genus observed direct nuclear division in its vegetative hyphae.

The work, presented in the following pages, deals with the cytological study of the vegetative hyphae of *Saprolegnia delica* Coker.

## Material and Methods

The culture of *Saprolegnia delica* Coker was obtained from Centraal Bureau voor Schimmelcultures, Baarn, Holland.

The material for the study of the vegetative mycelium was obtained by growing the fungus in 1 per cent solution of Merck's bacto-peptone for about 24 hours at 25°C. In living condition it was studied both with and without the help of vital dyes dissolved in Ringer's solution.*

For the cytological studies, the methods and technique employed were the same as used by the senior author in his studies on the genus *Pythium*[14].

## Observations on Living Material

*General observations.*

When the actively growing mycelium is examined under the high power of the microscope, there are seen in the broad hyaline hyphae a large number of minute rounded shining bodies, moving rapidly in the cytoplasm (Fig. 1). The number and size of these bodies vary in different parts of a hypha, being fewer and smaller at the tips as compared to those in the older portions. At some places their number is so large that nothing can be seen except these particles. Their reactions towards 1 % osmium tetraoxide, Sudan III, conc. sulphuric acid and ammonium molybdate, methyl alcohol, ether, benzene and chloroform go to show their lipoid nature. These fat particles have been observed in many fungi, *e.g.*, *Saprolegnia*[4], *Phytophthora*[1], *Pythium*[14], and *Achlya* and others[13].

Due to very little difference between the refractive index of the vacuolar sap and the cytoplasm it is difficult to distinguish the vacuolar system in a living hypha. Sometimes in the tips of certain hyphae small round or elliptical vacuoles become visible. This system becomes very distinct when the hyphae are treated with vital dyes.

The nuclei, each with an indistinct nucleolus, are seen, though rarely, in the form of oval or fusiform bodies (Fig. 1).

The mitochondria usually appear as filamentous, delicate and slender bodies of varying length lying mostly parallel to the longitudinal axis of the hypha (Fig. 1). They have nearly the same refractive index as the cytoplasm and move very sluggishly in it.

A study of the living hyphae also reveals in the vacuolar canal a number of corpuscles of various dimensions—2 to 4μ or more in diameter. (Fig. 1). Their refrigence is like that of mitochondria and they are found singly or in groups. They take up vacuolar dyes quickly but less intensely and are also stained vitally and

*Ringer's solution contains: Sodium chloride, 6 gms., potassium chloride, 0·075 gms., calcium chloride 0·1 gm., sodium bicarbonate 0·1 gm., and distilled water 1000 c.c.

post-vitally with cotton blue and Delafield's haematoxylin. On the other hand they are not stained with Sudan III or scarlet red and are insoluble in NaOH solution, alcohol, ether, xylol and acetone. The nature, origin and the function of these pre-existing corpuscles are not yet clear.

*Supra-vital staining.*

The vacuolar system becomes more clear and can be studied easily with the help of vacuolar dyes, *viz.*, neutral red, cresyl blue, nile blue, toluidine blue and methylene blue, dissolved in Ringer's solution in different concentrations varying from 1 mg. per cent to 10 mg. per cent. In each case the vacuoles take up the dyes in dilute solutions very quickly, neutral red giving them a reddish orange colour while the colour with the other dyes varies from blue to violet. Of all the dyes neutral red penetrates more quickly and is less injurious than others. In the tips of growing hyphae, treated with vital dyes, the origin and evolution of the vacuolar system can be seen. The vacuoles arise *de novo* and first appear as tiny isolated bodies coloured intensely under the action of the vital dyes. They collide with each other giving rise to bigger ones by fusion, the latter fuse and sometimes give an appearance of a network (Fig. 3) or give rise to a chain of round and ellipsoidal vacuoles (Fig. 2). In the older portion of the hyphæ big vacuoles by fusion give rise to a regular vacuolar canal. The colour of the smaller vacuoles at the tips is more intense than that of the bigger vacuoles due to higher concentration of the vacuolar sap in the former. As the sap becomes more diluted the intensity of the colour of the dye decreases. In a branching hypha sometimes a portion of the vacuolar canal passes out into the daughter hypha, in the tips of which small vacuoles arising *de novo* are also seen (Fig. 4).

As soon as a vacuolar dye enters into the vacuoles there appear a large number of deeply stained tiny particles showing Brownian movements. They collide with each other giving rise to bigger and fewer corpuscles. As they increase in size by fusion they show sluggish movement and in some cases they come to lie touching the periphery of the vacuoles (Fig. 4). These corpuscles are the result of precipitation of the colloidal substance of the vacuolar sap under the action of the vital dye and are known as the vacuolar precipitates. These corpuscles cannot be preserved by any fixative and are soluble in alcohol, formol, potassium dichromate 3%, ether and acetone. The two tests used by Guilliermond[6] to ascertain the metachromatic nature of the vacuolar precipitates give negative results in the case of these corpuscles indicating in them the absence of metachromatin and volutin.

The mitochondria are stained in the living condition with either janus green Höcht B or dahlia violet dissolved in Ringer's solution in very low concentrations

(·0001 gm. per cent). It takes about 10—15 minutes to stain the mitochondria with these dyes, which stain them in phases preceding death due to their toxicity. With janus green they take up a sky blue colour while dahlia violet imparts them a light violet tinge. After being stained with janus green their structure becomes more clear (Fig. 5) and is the same as already described when no dye is employed. If they are constantly watched, they are seen fragmenting, each fragment later on becomes vesiculised (Fig. 5). Dahlia violet seems to be more toxic than janus green since in equal concentrations it causes earlier fragmentation and vesiculisation of the mitochondria (Fig. 6). After a short time the blue colour of the janus green in the hyphae changes to pink. This phenomenon has been observed in many fungi[13], [14] and is due to the formation of a reduction product of the dye.

By using a mixture of neutral red and janus green Höcht B the vacuoles and the mitochondria are simultaneously stained, the former take up the neutral red while the latter are stained by janus green. This shows clearly that the mitochondrial and the vacuolar systems are two distinct and separate systems.

*Intra-vital staining.*

a. Liquid medium.

To stain the vacuolar system intravitally the fungus was grown in 1% bacto-peptone solution to which the vital dyes were added in concentrations ranging from 0·25 mg.% to 5 mg.%. Height of the solution (usually 25 cc.) in tubes (15 × 2·5 cm.) was about 6 cms. The fungus for inoculum was grown on bacto-peptone agar (Bacto-peptone 1 gm., agar 20 gms., and distilled water 1000 cc.).

TABLE I

*Relative heights in cms. to which the fungus colonies rise in 1% bacto-peptone solution to which vital dyes were added in different concentrations. Time of incubation=4 days. Temperature=25°C.*

| Quantity of dye per 100 c.c of 1% peptone solution. | Control without any dye. | Neutral red. | Cresyl blue. | Toluidine blue. | Methylene blue. | Nile blue. |
|---|---|---|---|---|---|---|
| | 5·0 | ... | ... | ... | ... | ... |
| 0·25 mg. | ... | 5·0 | 5·0 | 4·5 | 2·3 | 2·0 |
| 0·5 mg. | ... | 5·0 | 4·5 | 3·9 | 1·5 | 1·0 |
| 1 mg. | ... | 5·0 | 4·0 | 3·2 | No growth | No growth |
| 2 mgs. | ... | 4·3 | 3·0 | 1·8 | No growth | No growth |
| 3 mgs. | ... | 4·0 | 1·9 | 1·0 | No growth | No growth |
| 4 mgs. | ... | 3·5 | No growth | No growth | No growth | No growth |
| 5 mgs. | ... | 2·5 | No growth | No growth | No growth | No growth |

The results summarised in Table I show that neutral red is the least toxic of all other dyes, as regards growth of the fungus, and then come cresyl blue, toluidine blue, methylene blue and nile blue in order of increasing toxicity. Another conclusion, to which one can arrive at, is that the increasing doses of a particular dye retard the growth. This has been the experience of Guilliermond[7], Saksena[14] and Murdia[13] also.

As regards the colouration of the vacuoles it is seen that the vacuoles are coloured in all cases where there is growth in the presence of neutral red and toluidine blue only. The intensity of the colour decreases with the decrease in concentration of the dye till in 0·25 mg. % of neutral red there is a diffuse colouration. It is curious to state that the other three dyes, which colour the vacuoles when the hyphae are supravitally stained with them, do not accumulate inside the vacuoles in the intravital staining and that the colour of the dyes in the medium in the lower part of the tubes disappears with the growth of the fungus. The colour of the neutral red in the medium inoculated with the fungus also disappears but this occurs after several days. Probably these dyes are reduced by the growth of the fungus. Guilliermond[9] (p. 296) has also observed this phenomenon.

When the hyphae, stained intravitally in neutral red, are examined, they reveal the same phenomenon as seen with supravital staining. The early stages of the vacuolar system are observed in the tips of the growing hyphae while in older but living portions the vacuolar precipitates and the pre-existing corpuscles are also seen in the vacuolar canal.

If 1% solution of Merck's bacto-peptone, to which neutral red is added in different concentrations, is inoculated with the fungus growing on soya-decoction-agar* for 8 days, there are seen in addition to the vacuolar precipitates and pre-existing corpuscles some other structures known as sphaerocrystals. They appear in the vacuolar canal of the hyphæ growing in solutions where the concentration of neutral red is 0·6 mg. % to 2 mgs. %, while in lower concentrations they are absent. Small sphaerocrystals usually appear on the second day when young hyphae emerge out from the inoculum. They increase in size and number on the 3rd and the 4th day. They are seen in all parts of hyphae, *i.e.*, in their extremities as well as in older but living portions. They are not localised only in the portions of mycelium which are in contact with the fragments of the agar or a little distance from it as has been reported by Guilliermond[8] but are found even in the hyphae that are far away from the inoculum. The size of these sphaerocrystals varies from small granular bodies to big bodies with a number of intermediates between them. They are often welded together by the face of their contact in 2-3 or large numbers.

* Soya-decoction is prepared by boiling 25 grains of soya bean in 500 cc. of distilled water on a small flame for one hour.

A very distinct hilum and radiating striations (Fig. 7) are observed in each sphaerocrystal, which shows the phenomenon of a black cross (Fig. 8) in polarised light. They are readily soluble in fat solvents, *viz.*, absolute alcohol, KOH solution 10%, ether, benzene and formol but are insoluble in acetone. If neutral red is omitted from the medium the sphaerocystals do not appear.

b. Solid medium.

Nutrient media containing 20 grams of agar, 1 gm. of bacto-peptone, 1000 cc. of distilled water and neutral red (1—5 mgs.% concentrations) were prepared, and were inoculated with the fungus. It was observed that the growth was retarded with the increasing doses of the dye and the vacuoles too remained uncoloured, though the vacuolar precipitates were observed. These results are in accord with those of Allain[1] who also failed to get coloured vacuoles but are in variance with those of the senior author[14] who succeeded in getting the coloured vacuoles with precipitates in the four species of *Pythium*, under similar conditions on solid media.

## Observations on Fixed Material

Of all the fixatives tried to fix mitochondria Helly's liquid and corrosive sublimate-formol solution were found to be most satisfactory. Mitochondria are stained black while the cytoplasm remains nearly colourless. Long filamentous mitochondria (Figs. 9, 10) are seen lying in the filaments. They are also observed in the process of fragmentation and vesiculisation (Fig. 11).

For the fixation of the nuclei Flemming's weak solution modified by Saksena[14] (p. 159) gave the most satisfactory results. The main portion of the nucleus consists of a central body which takes a dark stain with iron-alum haematoxylin. Surrounding this is a layer of nucleohyaloplasm which is bounded externally by a distinct nuclear membrane. No nuclear division was observed in the hyphae. Chromatin threads reported by various workers were not seen though mounds of chromatin were visible on the inner side of the nuclear membrane (Fig. 12).

The authors thank Dr. S. B. Dutt of the Chemistry Department, Allahabad University, for kindly allowing them to make use of his microscope fitted with the apparatus for polarised light and also for taking the micro-photograph of the sphaerocrystals.

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## Explanation of Figures

Abbreviations used :

C = Cytoplasm. F = Fat particles. H = Hilum. M = Mitochondrium. $M_1$ = Fragmenting mitochondrium. $M_2$ = Vesiculised mitochondrium. N = Nucleus. $N_1$ = Nucleolus. $N_2$ = Nuclear membrane. P = Pre-existing corpuscles. S = Sphaerocrystals. V = Vacuole. $V_1$ = Vacuolar canal. $V_2$ = Vacuolar precipitates. $V_3$ = Vacuolar canaliculae—net work.

Fig. 1. A hypha without being treated with a vital dye, showing fat particles, pre-existing corpuscles, mitochondria and a nucleus (×2135).

Fig. 2. A hypha treated with neutral red solution showing the initial stages of the vacuolar system in the form of rounded and ellipsoidal vacuoles. Some vacuoles are seen arising *de novo* at the tip of the hypha. (×2135).

Fig. 3. A hypha treated with neutral red solution showing the initial stages of the vacuolar system in the form of a reticulum (×2135).

Fig. 4. A portion of a hypha with a small branch treated with neutral red, showing the origin of the vacuolar system in the branch, and also the passing of a part of the vacuolar canal of the parent hypha into the daughter hypha (×2135).

Fig. 5. A portion of hypha after being treated with janus green Höcht B for about 15 minutes showing filamentous mitochondria. Some of them are in the process of fragmentation and vesiculisation (×2135).

Fig. 6. A portion of the hypha after being treated with dahlia violet for about 20 minutes. Nearly all the filamentous mitochondria have become vesiculised (×2135).

Fig. 7 A portion of hypha grown in 1% bacto-peptone solution to which 2 mg.% neutral red was added, showing sphaerocrystals in the vacuolar canal (×2135).

Fig. 8. A micro-photograph of sphaerocrystals appearing as black crosses in polarised light (×630).

Fig. 9. A hypha fixed in Helly's liquid and stained with iron-alum haematoxylin showing filamentous mitochondria and nuclei (×950)

Fig. 10. A portion of hypha fixed in Sublime-formol and stained with iron-alum haematoxylin showing filamentous mitochondria (×2135).

Fig. 11. A portion of hypha fixed in Helly's liquid and stained with iron-alum haematoxylin showing vesiculisation of its mitochondria (×2135).

Fig. 12. A portion of hypha fixed in Flemming's weak solution modified by Saksena and stained with iron-alum haematoxylin showing nuclei (×2135).

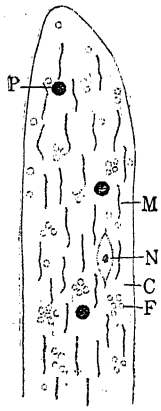
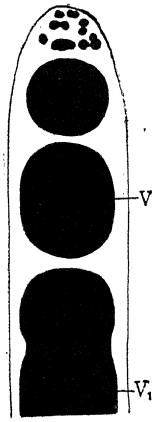



Fig. 1

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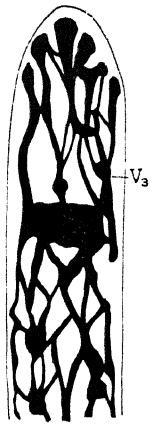


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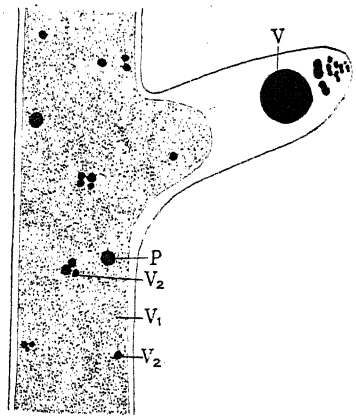


Fig. 4

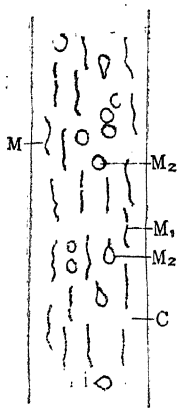


Fig. 5

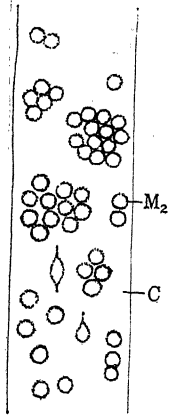


Fig. 6

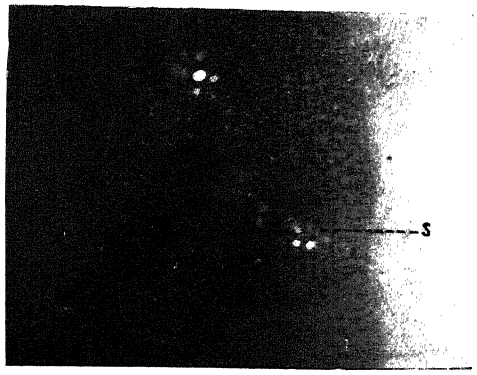


Fig. 8

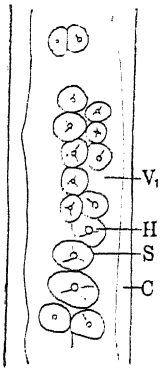


Fig. 7

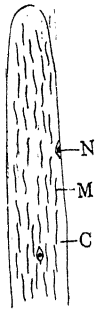


Fig. 9

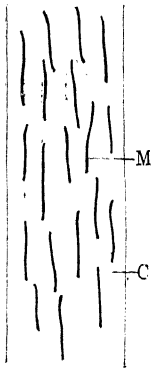


Fig 10

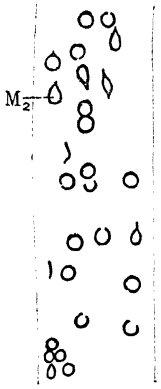


Fig. 11

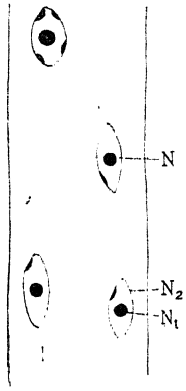


Fig. 12

# INSTRUCTIONS TO CONTRIBUTORS

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